Dual enzymatic activity of the pathogenesis-related protein TcPR-4 from *Theobroma cacao*: ribonuclease and Ca^{+2} and Mg^{+2} dependent deoxyribonuclease activities

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The class 4 pathogenesis-related proteins (PR4) are classified as chitinases and contain a conserved Barwin domain. The *TcPR-4b* cDNA identified from a library of *Theobroma cacao* L. pod (genotype TSH1188) infected by *Moniliophthora perniciosa* also presents the Barwin domain with six conserved cysteine residues, but lacks the chitin-binding site and for this reason was classified as class II PR4. The *TcPR-4b* gene was cloned into pET28a and the resulting in frame fusion plasmid was used to transform *Escherichia coli* Roseta (DE3) for protein expression. The expression of the TcPR-4b recombinant protein was induced by 0.4 mM isopropyl-β-D-thio-galactoside and purified by immobilized metal affinity chromatography with TALON® Metal Affinity Resin. To determine the DNase activity of the purified recombinant TcPR-4b protein, 1 µg of purified pGEM-T® Easy Vector DNA was incubated with different protein amounts (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg) in the presence or absence of 10 mM of $MgCl_2$ or 1 mM of $CaCl_2$ overnight at room temperature. RNase activity of recombinant TcPR-4b was performed using different protein amounts (5, 10, 15, 20 and 25 µg) incubated for 30 min with 5 µg of RNA extracted from *Solanum lycopersicum* leaves. The reaction products were analyzed in 1.5% agarose electrophoresis gel. The TcPR-4b protein recombinant showed both DNase and RNase activity. DNase activity was observed only in the presence of Mg^{+2} and Ca^{+2} ions. The results of this study suggest that TcPR-4b may act as nuclease during the infection of cacao plants with *M. perniciosa*.
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